Photo-stability of water-soluble quantum dots in living cells

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Hydrophilic photo luminescent semiconductor quantum dots (QDs) are novel nanometer-size probes, which may have potential using in bio-imaging for biological objects. In this work, the photo-stability of these QDs in two kinds of living cells was studied, compared with conventional biological probes such as fluorescein isothiocyanate (FITC) and green fluorescence protein (GFP). It was found that the concentration of QDs in living cells is the dominant factor for its photo-stability in biological environment. When the concentration of the intracellular QDs was high, the QDs show good photo-stability that is much better than the organic fluorescent probes. However when its concentration was low, the QDs also can be photo-bleached quickly. Thus the reaching of the certain concentration level is the critical condition for QDs in the application of bio-imaging.

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Fluorescence technique has been widely used in biomedical study, such as diagnosis and probe staining. The common fluorescence probes are organic fluorophores, such as fluorescein isothiocyanate (FITC) and rhodamine. Generally these organic dyes have narrow absorption bands and broad emission spectra, their photo stability is also poor[1].

Recently, hydrophilic photo luminescent semiconductor quantum dots (QDs) are novel nanometer-size probes, which may have potential using in bio–imaging for biological objects. These QDs are believed to have advantages as compared with conventional organic fluorescent probes, that is, higher photo-stability, stronger emission with narrow spectral width and changeable emission peak wavelength controlled by the size of dots[1,2].

Although QDs have higher photo-stability than the organic fluorescent probes[3], some works found that the QDs also can be photobleached[4,5]. In this work, the photo-stability of these QDs in two kinds of living cells was studied in comparison with conventional biological probes such as FITC and green fluorescence protein (GFP). We found that the QDs still can be photo-bleached in living cells, and the important parameter concerned to the photobleaching is the intracellular concentration of QDs.

The water-soluble CdTe QDs were used here. It was producted in hydrothermal synthesis. Briefly, NaHTe was prepared by the reaction of sodium borohydride and tellurium. $CdCl_2$ and thioglycolic acid were dissolved in water followed by adjusting pH to 9.0. NaHTe solution was added to above precursor solution, which was put into a Teflon-lined stainless steel autoclave. The reaction was carried out at 200 °C and then cooled to the ambient temperature[6]. The obtained QDs have the luminescence band centered on 600 nm.

In this work, two different cell lines, euglena gracilis and human embryonic kidney cell cultures (HEK 293 cells) were chosen as cell modes to study the photostability of QDs in living system. After being incubated with QDs of 0.1–0.2 mg/ml and then washed to remove un-bound QDs, the cells of euglena gracilis and HEK 293 were measured respectively by laser scanning confocal microscope (Olympus, FV-300) with the excitation of 488 nm from the Ar^+ laser and the acquiring band filter of 585–630 nm to obtain the luminescence images of QDs in cells. The confocal images were shown in Fig. 1, that the intracellular content of QDs is quite different for two cell lines. The bright image in the cells of euglena gracilis reflects that the concentration of QDs is high, the weak intensities in cytoplasm of 293 cells demonstrate that the concentration of QDs is lower. Focusing the laser on selected part of the cell, the photobleaching of QDs at that spot could be measured. For evaluating the photo-stability of the QDs in cells, the photobleaching of the chlorophyll (fluorescence emission at 690 nm) in euglena gracilis and FITC as well as GFP in 293 cells was measured in parallel for comparison. The half-life time of QDs and these fluoresceins were shown in Table 1. We found that, when the concentration of QDs is higher such as in euglena gracilis, the QDs are extremely stable even comparing with the native fluorescein of chlorophyll. When its concentration is lower such as in 293 cells, the life-time of QDs dramatically reduces comparing with that in Euglena Gracilis, but still is comparable with that of GFP and much longer that that of FITC. So, the QDs can be believed stable with the condition that its concentration should be not too low.

The QDs' distribution in 293 cells is totally un-uniform (Fig. 1). The luminscene intensities are directly proportional to the concentration of QDs. Thus in 293 cells the luminescence image reflects the concentration distribution of QDs in the cell. When laser was focused on

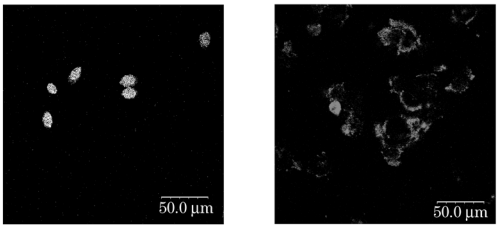

Fig. 1. Confocal image of euglena gracilis (left) and 293 cells (right) labeled with CdTe QDs. Excitation : 488 nm; imaging acquiring filter: 585–630 nm.

Table 1. Half Lifetime of QDs and Fluoresceins in Two Kinds of Cells Measured in Photobleaching Courses. The Power Density of Laser Irradiation Is 8×10^4 W/cm^2

	QDs (s)	Chlorophyll (s)	GFP (s)	FITC (s)
Euglena Gracilis	20.099	5.555		
293 Cells	1.7668		1.8299	0.1578

different small area (1 μm), the photobleaching can be detected for different area with different concentration of QDs, in which the initial luminescence intensity represents the QDs concentration of that part. The concentration effect of QDs on photobleaching is shown in Fig. 2, that the photobleaching is slow in high QDs' concentration area, but very fast in the part with very low QDs' concentration. The concentration effect on QDs' photobleaching was directly demonstrated in Fig. 2. In Fig. 2, the numbers in label show the initial luminescent intensitys of the photobleaching curves, which are in direct proportion to the concentration of QDs in small area. The photobleaching curve of QDs excited at wavelength 488 nm by point scan mode of Olympus FV300 Confocal Microscope. The power density of laser irradiation is 8×10^4 W/cm^2.

Why the QDs still can be photobleached? Maybe the photooxidation also happen, something like that the photooxidation of photosensitizer in photodynamic therapy (PDT)[7]. The oxygen was suspected as the major factor in the photostability of QDs[8]. Here the oxygen effect on QDs photobleaching was experimentally explored for the first time. The results, shown in Fig. 3, convince the

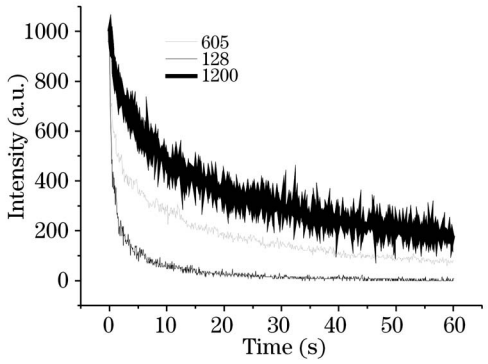

Fig. 2. Concentration effect: the relation between the concentration and photobleaching of the QDs in 293 cells.

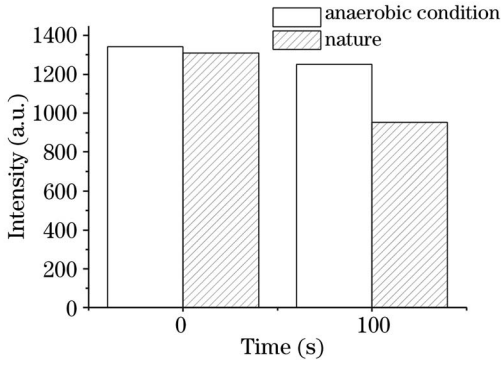

Fig. 3. Oxygen effect: the photobleaching of water-solution QDs (0.07 mg/mL), which irradiated by 532–nm Nd:YAG laser with power of 250 mW for 150 s. The white column shows the photobleaching of the QDs in anaerobic condition (bubbled with pure $N_2$for 30 min). The gray one shows the photobleaching of QDs in air condition.

oxygen effect. When QDs aqueous solution was bubbled with N_2 gas for 30 minutes, the photobleaching was effectively eliminated, comparing with that in air condition.

In conclusion, the concentration of QDs in living cells is the dominant factor for its photo-stability in biological environment. The reaching of the certain concentration level is the critical condition for QDs in the application of bio-imaging. J. Chen is the author to whom the correspondence should be addressed, his e-mail address is jychen@fudan.edu.cn.

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